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PLANT REGENERATION FROM *IN VITRO* AXILLARY BUD OF *OCIMUM BASILICUM* L. - AN IMPORTANT MEDICINAL PLANT

LEELAVATHI D[1] AND NARENDRA KUPPAN[2*]

1: Head of Department of Botany, MES Degree College of Science, Bangalore

2: DOS in Botany, University of Mysore, Mysore

***Corresponding Author: E Mail: narendrakuppan@gmail.com**

ABSTRACT

The prime objective of the present investigation was to develop a repeatable protocol for rapid clonal multiplication using *in vitro* axillary bud of *Ocimum basilicum.* On MS basal medium supplemented with BAP (8.88 µM).It induced maximum number of multiple shoots with 85% response. Sub-cultured on the same medium to obtain more number of shoots. These healthy multiple shoots developed root on the same medium thus avoiding an additional step of *in vitro* rooting and later was subjected to hardening. Rooted plantlets were made to acclimatize, hardened and gradually transferred to the field with 80% survival rate.

Keywords: *Ocimum basilicum*, Multiple Shoots, MS Medium BAP

INTRODUCTION

Aromatic plants are economically important because of the essential oil they produce. In the event of repeated failure of essential crops due to diseases and vagaries of weather aromatic plants can provide alternate and regular source of income to farmers. There is a strong need of promoting and popularising this wealth among the farmers entrepreneur and pharmaceutical industry. Further, the number of aromatic crops in commercial cultivation is limited and information available on their multiplication and cultivation technique is scanty. Under such circumstances tissue culture technique becomes more relevant in handling large scale multiplication and crop specific problems.

Ocimum basilicum (Sweet basil) is an aromatic plant belongs to the class Dicotyledon order Tubiflorae family Lamiaceae is a popularly known as Sweet basil ***Ocimum basilicum*** L. is an annual

aromatic herb **(Figure 7)** consisting of 160 species.

Although these aromatic plants can be propagated vegetatively, the poor rooting ability of the stem cuttings, as well as the lack of selected clones, restrain industrial exploitations. Further, limited tissue culture work has been done on aromatic plants to date as suggested by **[1]**. Therefore, it is imperative to develop efficient protocols using

Explants, Such aromatic plants are gift of nature it should be protected and Propagated **[2].**

The relevance of the present work is an attempt to produce large number of disease free planting material available to the farmers at an affordable price and also the establishment of protocol for long time conservation of aromatic plants to be used on cultivation programmes.

MATERIAL AND METHODS

The axillary bud measuring 0.75 cm with stem was excised from 25 days old ***in vitro*** plants of ***Ocimum basilicum*** **(Figure 1)** and cultured on MSBM fortified with different concentrations of BAP ranging from 4.44 µM, 6.66µM, 8.88 µM, 11.11 µM, 13.32 µM: and KN ranging from 4.64 µM, 6.96 µM, 9.28 µM, 11.60 µM, 13.92 µM separately to study their effect on axillary bud multiplication **(Table 1)**. Shoot initiation was observed from axillary bud explant after 7 days of culture with 2-3 leaves **(Figure 2)** on all the concentrations of growth regulators tried with varying percentage (46-85 %) of response **(Table 1, Graph 1)**. The highest (85) and lowest (46%) percentage of response was observed on MSBM + BAP (8.88 µM) and MSBM + KN (13.92 µM) respectively. After 21 days of culture 1-2 multiple shoots were noticed **(Figure 3)** and **3-7** elongated multiple shoots (s) were observed after 28 days of culture. After 35 days of culture, the shoots were subcultured on the same medium to obtain more number of multiple shoots. After 21 days of subculture, 20 multiple shoots were observed **(Figure 5)** on MSBM + BAP (8.88 µM) and 20-30 multiple shoots were observed **(Figure 6)** after 42 days of subculture. 30 elongated multiple shoots which attained the height of 6-8 cms **(Figure 7)** was observed after 63 days of subculture with well developed roots **(Figure 7)**. These 30 healthy multiple shoots developed root on the same medium thus avoiding an additional step of *in vitro* rooting **(Figure 8)** and later was subjected to hardening **(Figure 9)**. In the present investigation, the statistical analysis of the data revealed highly significant differences existing between and within the treatments. The mean number of shoots per explant ranged from 16.10 to 30.00 **(Table 1-2, Figure 10)**. The highest mean number 30.00

was observed on MSBM + BAP (8.88 µM) and the lowest mean number 16.10 on MSBM + Kn (13.92 µM) respectively.

RESULT AND DISSCUSION

The success of tissue culture protocols ultimately depends on the plant chosen, size of the explant, age and the manner in which it is cultured **[3]**. In general, juvenile explants such as apical bud, axillary bud, embryos, cotyledon and hypocotyl explants from seedlings are more responsive than the other tissues. Best results were achieved when they are from juvenile parts of the plants as suggested by **[4]**.

In the present study, *in vitro* vegetative propagule, axillary bud of ***O. basilicum*** was cultured supplemented with various combinations and concentrations of growth regulators BAP, Kn, IAA, NAA, IBA and 2, 4-D separately It was found in the present study, that MS basal medium supplemented with BAP (8.88 µM) and NAA (2.68 µM); and MSBM with BAP (8.88 µM) was the best medium for *in vitro* axillary bud initiation and shoot multiplication of ***L. angustifolia*** and ***O. basilicum*****.** These findings don't coincide with the findings of **[5-6]**, but coincide with findings of **[2, 7]**.

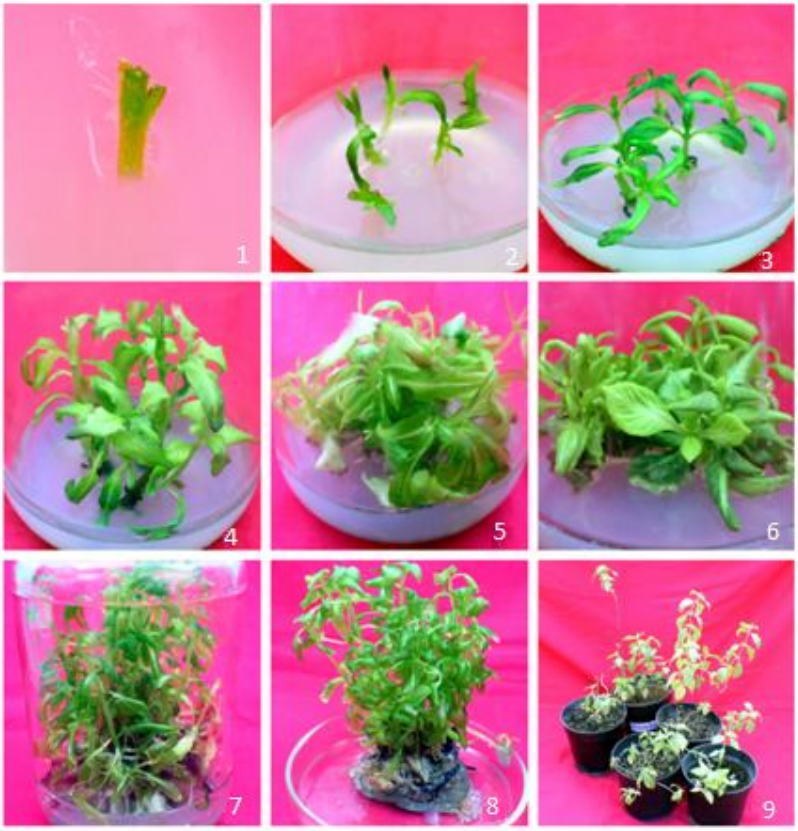

Figure 1-9: Various Stages of Growth of Axillary Bud of *Ocimum basilicum* in Vitro Nutrient Medium

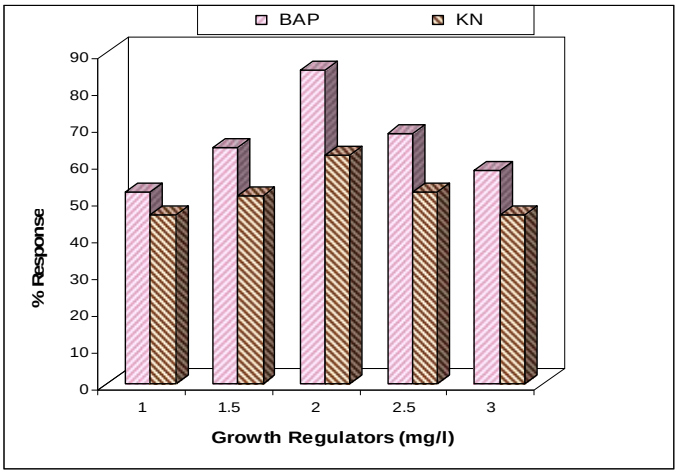

Figure 10: Effect of Different Concentrations of Growth Regulators on Initiation and Multiplication of Shoots From in Vitro Axillary Bud Explant of *Ocimum basilicum*

Table 1: Effect of Different Concentrations of Growth Regulators for Initiation and Multiplication of Shoots From *in vitro* Axillary Bud Explant of *Ocimum basilicum*

Basal media	BAP (µM)	BAP (mg/l)	Response (%)	No. of shoots /Explant X *± SD
MS	4.44	1.0	52	18.40 ± 1.01
MS	6.66	1.5	64	22.50 ± 2.33
MS	8.88	2.0	85	30.00 ± 2.52
MS	11.11	2.5	68	24.00 ± 2.93
MS	13.32	3.0	58	20.30 ± 1.41
	Kinetin (µM)	Kinetin (mg/l)		
MS	4.64	1.0	46	16.10 ± 1.75
MS	6.96	1.5	51	18.10 ± 1.57
MS	9.28	2.0	62	22.00 ± 2.09
MS	11.60	2.5	52	18.30 ± 1.55
MS	13.92	3.0	46	16.10 ± 1.81

Table 2: Anova Table (Number of Shoots/Explant)

SV	DF	SS	MSS	F_{cal} ratio	F_{tab}value**	CD
Treatment	9	1624.56	180.50	41. 68	2.00	3.98
Errors	90	389.80	4.33			
Total	99	2014.36				

Note: *: Mean of 10 Replication; **: Significance F Value @ 5 % Level

CONCLUSION

In the present study, it was observed that MSBM + BAP (8.88 µM) was the best medium for axillary bud intiation and shoot multiplication. Further, it was significantly superior than the other concentrations of growth regulators tested with respect to multiple shoots formation.

REFERENCES

[1] Calvo MC and Segura J, *In vitro* propagation of Lavender, Hort. Sci., 24 (2), 1989, 375-376.

[2] Leelavathi D and Kuppan N, Protocol For Rapid Clonal Multiplication Using *In Vitro* Apical Bud Of *Lavandula Angustifolia*, IOSR J. Pharm. and Biological Sci., (IOSR-JPBS) 7 (3), 2013, 96-98

[3] George EF and Sherrington PD, Plant propagation by tissue culture, Handbook and dictionary of commercial laboratories Exegetcs Ltd., England, 1984.

[4] Bonga JM, Clonal propagation of mature trees: Problems and possible solutions. In: Bonga, J.M. and Durzan, D. J. (eds), Cell and Tissue Culture in Forestry, Martins Nighoff publishers, Dordrecht, 1987, 249-271.

[5] Panizza M and Tognoni F, Micropropagation of Lavandin (Lavandula officinalis chaix x Lavandula latifolia villars c.v. Grosso) In: Bajaj, y.p.s. (ED.), Biotechnology in Agriculture and Forestry: High-tech and micropropagation 111, springer-verlag Berlin Heidelberg, 19, 1992, 295-305

[6] Miguel Carlos Sanchez-Gras and Maria Del Carmen Calvo, Micropropagation of *Lavandula latifolia* through nodal bud culture of matured plants, Plant cell, Tissue and Organ Culture, 45, 1996, 259-261.

[7] Leelavathi D, Ex-Situ Conservation Of *Lavandula Angustifolia* Using *In Vitro* Techinques, Lake 2010: Wetlands, Biodiversity and change 22 -24th, December 2010, IISC Bangalore.